ISSN: 3048-6181 www.wjphr.org 2026, Volume 3, Issue 1. Page: 17-25.

World Journal of
Pharmaceutical and Healthcare Research

Research Article

Impact Factor 5.429

TRADITIONAL FERMENTED FOOD AND THEIR STARTER CULTURE IN ETHIOPIA

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Article Info
Article Received: 04 January 2026,
Article Revised: 22 January 2026,
Published on: 01 February 2026.

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How to cite this Article: *Meskerem Elfu. (2026). Traditional Fermented Food And Their Starter Culture In Ethiopia. World Journal of Pharmaceutical and Healthcare Research, 3(1), 17–25.

ABSTRACT

Starter cultures are organisms which are used to initiate a fermentation process. It can be obtained either as pure culture from a commercial laboratory or from a portion of previously cultured product. The microorganisms are selected for their ability to produce lactic acid for crude production and a low pH to prevent spoilage, produce metabolites that give desirable flavors, or produce enzymes that mature the product. In Ethiopia traditionally fermented foods and beverages are consumed in different occasions such as marriages, at festivals, social gatherings and ceremonies. In Ethiopia fermentation takes place traditionally without using modified starter culture. During fermentation different pathogenic and food spoilage organisms enter in the food and they may cause disease to consumers. This problem may be solved by using modified starter culture which inhibit the growth of pathogenic and food spoilage organisms. Many developing countries use modified starter culture to ferment there foods. This review focuses on use of starter culture, fermented food by starter culture and organisms used as starter culture in Ethiopia and developing countries.

1. INTRODUCTION

Lactic acid bacteria (LAB) are the primary microorganisms used to ferment maize, sorghum or millet based foods that are processed in West Africa. Fermentation contributes to desirable changes in taste, flavor, acidity, digestibility and texture in fermented food products. Similar to other fermented cereal foods that are available in Africa, these products suffer from, inconsistent quality (Soro *et al.,* 2014).

In Ethiopia traditionally fermented foods and beverages are consumed in different occasions such as marriages, at festivals, social gatherings and ceremonies. The variety of foods and beverages processed and consumed among the various ethnic groups are manifestation of this diversity. Some of the food items may be consumed in their raw forms, processing of one type or another is usually a rule than an exceptions. This usually includes salting and drying, boiling, roasting, frying, baking, cooking, fermenting or various combinations of these. In Ethiopia fermentation is usually natural, with no defined starter cultures used to initiate it. In most cases, this is made possible through the proliferation of the initial food flora, with microbial succession determined by ambient temperatures and chemical changes in the fermenting food (Mogesse, 2006).

According to Leroy (2006) using Functional starter cultures offer an additional functionality compared to classical starter cultures and represent a way of improving and optimizing the substrate fermentation process and achieving tastier, safer and healthier products. Commercial starter cultures are supplied in concentrated form by freeze-drying, vacuum-drying, spray- drying, drum-drying, fluidized bed-drying or air-drying. The use of dried starter cultures has been shown to be of great benefit by small-scale processing units in Senegal (Soyo *et al.,* 2014).

In Ethiopia preparation of many indigenous or traditional fermented foods and beverages is still a household art. In addition to these traditional foods and traditional food processing techniques form part of the culture of the people. Traditional food processing activities constitute a vital body of indigenous knowledge handed down from parent to child over several generations. Unfortunately, this vital body of indigenous knowledge is often undervalued. In this review, history of starter culture, the importance of starter culture, function of starter culture, organisms involved in starter culture, different types of Ethiopian fermented foods and their starter culture will be discussed.

2. History of Starter culture

The history of starter culture fermentation has a long tradition. At first, there was a purely empirical principle without the connection between metabolic activity of microorganisms and desired changes in the product (Sawitzki *et al*., 2009). Louis Pasteur was the first to recognize fermentation nature, describing fermentation as a microbial "life without air" (SKLM, 2010). In 1866 Pasture discovered microorganisms as sources for fermentation process. In 1892 Hansen started selling the first commercial starter culture for diary industry (Albrecht, 2013).

According to Susanna (2001) the first LAB meat starter culture, introduced as a pure culture of *Pediococcu scerevisiae*, was developed in 1955. At the same time, Niinivaara in 1955 applied *Micrococcus* to dry sausage production in Europe. The work of Niinivaara was continued by Nurmiin 1966 that combined *amicrococci* with *Lactobacillus plantarum*. Today, several companies provide Lactobacillus species, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Staphylococcus xylosus* or *Staphylococcus* carnosus strains in pure cultures or their mixtures for fermentation of meat. In 1972 the first international symposium of starter culture in Helsinki helped to get starters accepted by butchers and the meat industry (Albrecht, 2013).

Recent study shows that commercial starter cultures used to improve quality of artisanal dry fermented sausage in Argentina (Prpich *et al.,* 2015). Different kinds of commercially available functional starter culture used to initiate meat fermentation. The isolates used in meat fermentation were able to perform fermentation properly with various health benefits (Bevilacqua *et al*., 2015).

2.1. Definition of starter culture

A starter culture can be defined as a preparation of live microorganisms or their resting forms, whose, metabolic activities has desired effects in the fermentation substrate, the food. The fermentation may contain unavoidable residues from the culture substrate and additives that support the vitality and technological functionality of the microorganisms (Albrecht, 2013).

2.2. Advantages of starter culture

Using starter cultures in food fermentation have the various advantages. Bacterial starters have been produced for a variety of fermented products to improve their sensory and other quality characteristics (Saeed *et al.*, 2009). Consuming fermented foods has health benefits; this is due to the presence of beneficial organisms in the food (Panesar, 2011).

- Preservation,
- Lactic acid production,
- Reduction of hygienic risk,
- Ensuring constant high product quality,
- Control the development of color and flavor,
- Control fermentation time,
- Reduction of cost by reducing fermentation time,
- Assures production of products of high safety and sensory quality (Sawitzki *et al*., 2009).

2.3. Starter culture organisms

The most common organisms responsible for fermentation of food are acid forming bacteria such as genera LAB which include Lactococcus, Lactobacillus, Streptococcus, Pediococcus, Leuconostoc, Enterococcus, Aerococcus, Corynobacterium and Oenococcus. LAB that are important in food technology those of include genera Lactococcus, Lactobacillus, Pediococcus and Leuconostoc. Some yeast such as Saccharomyces and Molds such as Penicillin, Aspergillus and Botrytisare also involved during food fermentation due to the lactic acid they produce. LAB play key role in safety and acceptability of fermented foods (Adusulu and Awojobe, 2014).

2.4. Characteristics of starter culture organisms

The LAB a component of several fermented foods including dairy products have long been consumed by people. LAB is utilized as starter culture organisms in different developed countries (Chang *et al.,* 2010). They are a group of gram positive, non-spore forming, cocci or rods, catalase negative, non-motile and which produce lactic acid as the major end product during the fermentation of carbohydrates (Teshome, 2015).

2.4.1. Lactobacillus

Lactobacilli are described as Gram-positive, catalase-negative, non-sporing rods, whose length varies between 1.5 μm and 5 μm. They may also have a slender, curved or bend appearance and frequently are able to form chains. Colony morphology is also variable on agar plates, with some strains producing large round colonies and others producing small or irregular colonies. Most of the *lactobacilli* are mesophilic, but the genus contains also species that are psychrotrophic, thermoduric or thermophilic. Temperature optimum varies from 30 to 45ºC. Some species show high tolerance to salt, osmotic pressure and low water activity. Acid tolerance is a common feature of lactobacilli and most of the strains are able to grow at pH below 4.4. The optimum pH value for their growth is 5.5–6.5. Some strains are ethanol tolerant and bile-tolerant as well. Most of the species are aero-tolerant, but some of them require strict anaerobic conditions (Grocova *et al*., 2011).

2.4.2. Pediococcus

Pediococci are characterized as being Gram-positive, nonmotile, catalase-negative and aerobic to microaerophilic bacteria. Members of this genus are Homofermentative with glucose converted to either l- or dl-lactate. Pediococci are chemoorganotrophs and require complex growth factor and amino acid requirements. In addition, these are the only lactic acid bacteria that divide in two planes, which results in the formation of pairs, tetrads or large clumps of spherical cells required nicotinic acid, pantothenic acid and biotin, whereas none required thiamine, *p*-aminobenzoic acid, or cobalamine. Several amino acids (glutamic acid, valine, arginine, leucine and isoleucine) appear to be essential for growth (kenneth and charls, 2007).

2.4.3. Leuconostoc

Leuconostocs are generally spherical or lenticular cells (0,4μm to 0,5μm), occurring in pairs or chains; they are non-spore forming, Gram-positive, non-motile and catalase-negative (Ethiopian Standards Agency, 2012).

2.4.4. Streptococcus and Enterococus

The genus streptococcus and enterococcus is gram positive, spherical or ovoid, cells which are typically arranged in pairs or chains. They are non-spore forming, facultative anaerobic, catalase negative, homofermentative and have complex nutritional requirement. Widely distributed in mucosal surface of man and animals, including gastro intestinal tract, but some are found in soil, water, dairy products and other foods and on the plants (Hardie and whiley, 1997).

2.5. Homofermentative LAB

Ferment glucose with lactic acid as the primary by-product. Homofermentative LAB includes Lactococcus spp. that is used in dairy starter culture applications where the rapid development of lactic acid and reduced pH is desirable. Other homofermentative LAB include yogurt strains consisting of rods (Lactobacillus delbruckii subspecies bulgaricus, Lb. acidophilus) and cocci (Streptococcus salivarius subsp. thermophilus) and thermophilic strains that might be used in cheese (e.g., Lb. helveticus). Other homofermentativecocci that might be found in milk and dairy products, but are rarely used as starter cultures include other Streptococcus spp., Enterococcus, Pediococcus and Aerococcus (Cornell University, 2000).

2.6. Heterofermentative LAB

Ferment glucose with lactic acid, ethanol/acetic acid and carbon dioxide (CO2) as by- products. Testing for heterofermentative fermentation generally involves the detection of gas (e.g., CO2). With the exception of certain fermented milk products, heterofermentative LAB are rarely used as dairy starter cultures, although they are not uncommon in milk and dairy products. If allowed to grow to significant numbers, they can cause defects related to their acid and CO2 production, such as slits in hard cheeses or bloated packaging in other dairy products. Heterofermentative LAB includes Leuconostoc spp. (Gram-positive cocci) and Gram-positive rods such as Lactobacillus brevis, Lb. fermentum, and Lb. reuteri. Other Lactobacillus species are considered “facultatively” heterofermentative, meaning they will produce CO2 and other by-products only under certain conditions or from specific substrates. These strains would include Lb. plantarum, Lb. casei and Lb. curvatus (Cornell University, 2000).

2.7. Other Gas Producing Pathways:

Some LAB has the ability to produce gas from other substrates including citrate, gluconate and certain amino acids. Certain citrate fermenters are used in some dairy products to provide flavor (e.g., diacetyl). Leuconostoc mesenteroides sub-sp. cremoris and Lactococcus lactis sub-sp. Lactis biovardiacetylactis are often used to make products such as buttermilk, sour cream and cultured butter. These organisms can also occur as wild contaminants causing defects in dairy products where certain flavors and gas are not desirable (Cornell University, 2000).

2.8. Nature of starter culture organisms

Starter culture of LAB that initiates acidification of raw substrate has different natures. In general they are nonpathogenic to man and animals. They need fermentable carbohydrates for growth and produce lactic acid as a sole or main product from the energy yielding fermentation of sugars. In addition, they can produce antimicrobial substances including Bacteriocins that have ability to inhibit pathogenic and food spoilage bacteria (Anas, 2012, Desalegn, 2013 and Esayas, 2006). LAB contributes to the aroma and flavor of fermented products. They acidify the food, resulting in a tangy lactic acid taste, frequently exert proteolytic and lipolytic activities and produce aromatic compounds (Hati *et al.,* 2013).

2.9. Growth condition of starter culture organisms

According to Anon (2005) and Askale and kebede (2013) observations LAB genera are generally mesophilic microorganisms but can grow at temperatures ranging from 5°C-50C. Temperature is one factor which affects fermented food. pH is another factor which affects fermented foods. In most fermented foods increase in acidity is due to the formation of lactic acid produced by LAB present in food during storage periods (Anjum and Zahoor, 2007), in addition to these LAB are salt tolerant. Addition of salt in food inhibit the growth of pathogenic and food spoilage bacteria. Among this inoculum size and fermentation period also influence fermented foods. Addition of higher amount of inoculum size leads to the product become sour this is due action of LAB (Huseyin *et al.*, 2006). Different temperature tolerant LAB genera presented below.

Table 1: Growth temperature of LAB.

Genera	Temperature C
Pediococcus	15-45
Lactobacillus	4-45
Leuconostoc	15-30
Lactococcus	4-15
Entrococcus	4-45

2.10. Fermented foods and their starter organisms

Ethiopian traditionally fermented foods consist of different kinds of starter organisms to initiate the fermentation process. Organisms obtained from food substrates. Such as tella is Ethiopian fermented beverage its fermentation takes up to 15 days (Haymanot, 2011). To initiate the fermentation maltadded as starter culture malt is the source of organisms in tela fermentation. Ethiopian traditionally fermented foods and African fermented foods and their starter organisms summarized below.

2.11. Probiotics

Probiotics may be defined as "live microbial food ingredient that, when ingested in sufficient quantities, exert health benefits on the consumer". Probiotics are now emerged as an important category of food supplement and could be found in conventional, dietary supplements and medicinal foods (Sarkar, 2013). According to Pundir (2013) observation the probiotic affects the host animal by improving its microbial balance"" Probiotic bacteria may produce various compounds, which are inhibitory to the growth of pathogen, which include organic acids (lactic and acetic acids), bacteriocins and reuterin. According to Tomas (2002) *lactobacilli, Bafidobacteria and Streptococcus* are microorganisms which are proposed as probiotics for both the gastrointestinal and urogenital tracts. In addition to this Lab which produce bacteriocine are widely used in probiotic products for human and animal consumption to prevent pathogens growth in the intestinal tract. They cause reduced lactose intolerance alleviation of some diarrheas, lowered blood cholesterol, increased immune response and prevention of cancer.

2.12. Bacteriocins

Bacteriocins are substances of protein structure possessing antimicrobial activities they are produced during the growth of a great number of Gram (+) bacteria and Gram (-) bacteria. Although bacteriocins could be categorized as antibiotics, they are not. The major difference between bacteriocins and antibiotics is that bacteriocins restrict their activity to strains of species related to the producing species and particularly to strains of the same species. Antibiotics on the other hand, have a wider activity spectrum and even if their activity is restricted this does not show any preferential effect on closely related strains (Zacharof and Lovitt, 2012). Bacteriocine kill or inhibit the growth of other bacteria. Many lactic acid bacteria produce a high diversity of different bacteriocins. Though these bacteriocins are produced by LAB found in numerous fermented and non-fermented foods, nisin is currently the only bacteriocin widely used as a food preservative. Bacteriocins are isolated from foods such as meat and dairy products, which normally contain lactic acid bacteria (Cleveland *et al*., 2001). Bacteriocins are also useful in food and feed industry because of their antibacterial characteristics; moreover, bacteriocins can be used as bio preservatives in fermented foods (Narender *et al*., 2010).

Table 2: LAB used as starter culture.

Food	Starter organism	Reference
Siljo	*Lactobacillus*	Mogesse, 2006
Yogurt	*Lactococcus*	Kucerova *et al*., 2009
	Lactobacillus	
	Entrococcus	
	Streptococcus	
	Leuconostoc	
	Pediococcus	
Sea foods	*Staphylococcus*	Francois, 2010
	Clostridium	
	Leuconostoc	
	Tetra gonococcus	
	Staphylococcus	
Tella	*Lactobacillus*	Haymanot, 2011
Ice cream	*Lactobacillus*	Javed and Muhammad, 2011
	Bafidobacterium	
Bread	*Lactococcus*	Gracova *et al*., 2011
	Lactobacillus	
	Entrococcus	
	Streptococcus	
	Carnobacterium	
	Pediococus	
	Arerococcus	
	Oenococcus	
	Tetragenococcus	
	Vagococcus	
	Weisella	

Poultry product	*Lactobacillus*	Surachon*et al*., 2011
Borde	*Lactobacillus*	Kebede, 2013
	Pediococcus	
	Wessilelia	
	Entrococcus	
Sausage	*Lactobacillus*	Zdoleca*et al.,* 2013
	Staphylococcus	
	Pediococcus	
Butter and Buttermilk:	*Lactococcus*	Hati*et al*., 2013
	Leuconostoc	
Enjera	*Lactococcus*	Desiye, 2013
	Leuconostoc	
	Lactobacillus	
	Entrococcus	
	Pediococcus	
Cheese	*Lactobacillus*	Khalaileh and Ajo, 2013
	Bafidobacterium	
Cassava	*Lactobacillus plantarum*	Tefera *et al.,* 2014
	Leuconostoc mesenteroides	
kocho	*Lactococcus*	Tiruha *et al*., 2014
	Leuconostoc	
	Lactobacillus	
	Entrococcus	
	Pediococcus	
	Lactobacillus	

2.13. Starter culture in different Ethiopian and African fermented foods

In Ethiopia starter culture used to reduce fermentation period in enjera, borde, bread…etc. Types of starter culture is previous fermentation (ersho, tinsis) and maltno use of commercial starter culture in Ethiopia, but in Africa most type of food fermentation is by using commercial starter culture as presented in the Table 3.In Africa starter culture is used to reduce toxic level of the foods like cassava to reduce the content of cyanide level (Anon, 2005). The function of starter culture in Ethiopian and African food fermentation summarized below.

Table 3: Starter culture used foods and their role in fermentation.

Type of food	Function of starter culture	Type of starter used	Reference
Gari(cassava)	➢ Reduce processing time ➢ Reduce cyanide level ➢ Reduce pH	Commercial	Anon, 2005
Enjera	➢ Reduce fermentation period ➢ Leavening the butter of teff ➢ Produce flavor compounds ➢ Inhibit pathogenic microbes	Ersho or prefemented teff	Mogesse, 2006
Cheese	➢ Reduce pH ➢ Reduce fermentation time	Commercial	Kongo, 2013
Bread	➢ Extend shelf life ➢ Enhance aroma and flavor ➢ Prevent growth of fungi ➢ High quality product	Pre fermented dough	Muhialidin *et al*, 2013
Borde	➢ Quality	Prefermented and bikil	Kebede, 2013
	➢ Initiate fermentation		
Karibo	➢ Reduce fermentation period	Bikil	Abawari, 2013
Kocho	➢ Reduce pH	Traditionally prepared and previous fermented enset	Tiruha *et al*., 2014
Tella	➢ Reduce fermentation period ➢ Produce flavor compounds	Tinsis	Belay and Awraris, 2014

Meat	Produce tastier, safer and healthier product Initiate fermentation process ➢ Reduce microbial risks	Commercial starter	Bevilacqua *et al.*, 2015.
Sausage	➢ Quality product ➢ Histamine accumulation	Commercial starter culture	Wang *et al.*, 2015

LAB is pH and temperature tolerant as mentioned below. They tolerate lower pH and tolerate higher temperatures.

Table 4: Growth temperature and pH of LAB in different fermented foods.

Food	pH	Temperature °C	Reference
Siljo	3.9-6.0	18-22	Mogesse, 2006
Sour milk	4	30	Mogesse, 2006
Enjera	3.5-5.8	17-25	Mogesse, 2006
Kocho	4.2-6.5	15-50	Mogesse, 2006
Sour dough	3.3	15-45	Kockova *et al.,* 2011
Cheese	5.4	37	Khalaieh and Ajo, 2013

2.14. Raw materials for production of starter culture

Raw material required for the fermentation of food comes from the substrate itself. Enjera is one of traditionally fermented food in Ethiopia. It needs starter culture to be consumes as Enjera. Raw material needed for Enjera preparation is teff flour, water and ersho. Raw materials mixed together and incubated for 24 hr. Ersho used as starter culture to initiate fermentation. Ersho is previous fermented teff (Mogesse, 2006). Kocho is another Ethiopian fermented food obtained from enset. Enset is raw material for kocho preparation. Previous fermented enset used as starter culture (Tiruha *et al*., 2014). The bacteria use food as a substrate for their propagation. And also degrade cellulosic and hemicellulose materials, inhibit the growth of food spoilage and pathogenic microbes and preserve food for longer period. LAB inhibits the growth of food spoilage and pathogenic bacteria by producing bacteriocins (Chelule *et al*., 2010).

2.15. Development of starter culture

Starter cultures have been developed for use as inoculants in commercial fermentation processes in developed countries (Gerekova *et al*., 2011). Defined cultures are produced by pure culture maintenance and propagation under aseptic conditions. The process of starter culture development starts from isolating starter organisms from different sources like fermented foods and beverages. Isolates are characterized morphologically and biochemically. Strain preserved by using glycerol for longer periods. Preserved isolates precultured in MRS broth then separated by using centrifuge. Centrifuged culture dried by using lyophilizer and grounded finally the flour packed.

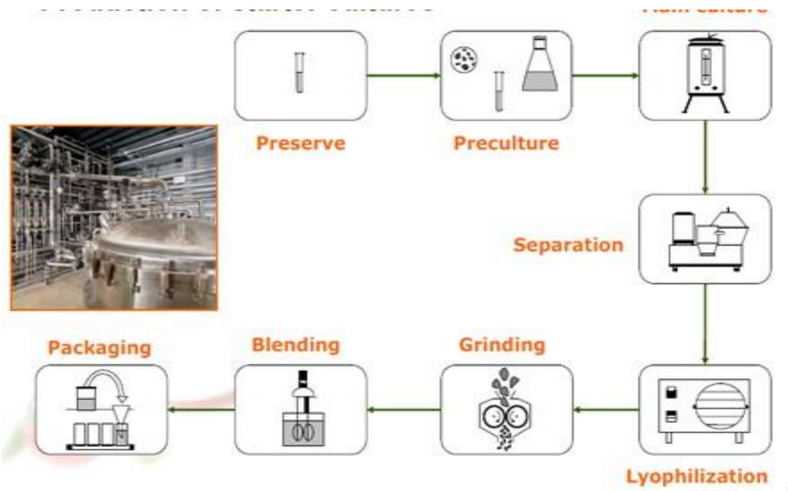

Figure 1: Development of starter culture.

They are generally marketed in a liquid or powdered form or else as a pressed cake. Addition of starter cultures is most often used when it is possible to inactivate the indigenous microbiota by heat treatment of the raw material, permitting the growth of only the added starter microorganisms (Guadalajara, 2010).

Figure 2: Commercially available starter cultures(A) liquid and (B) powdered form starter cultures.

3. Conclusion and future prospects

Addition of starter culture on different food substrates gives health benefits for consumers and improves nutritional value. Different studies define the use of starter cultures as a source of beneficial organisms for consumers, gives quality product for industries, reduce fermentation time, inhibit the growth of pathogenic microorganisms and food spoilage organisms and improve the nutritional value of food (Soro *et al*., 2014). In Ethiopia there is no use of starter culture fermentation takes place traditionally through the presence of microorganisms in the substrate (Haymanot, 2011).

Starter culture has a fundamental effect on the acidity, moisture content, texture and shelf life of the fermented food.Developed countries provide their nutritional value of fermented foods; receive quality product and probiotic organisms by using starter culture in their foods.

Public sectors should also come forward to support the agencies engaged in this field. With the help of microbiology techniques still a number of beneficial bacteria discovered for industrial uses. In order to have more potential starter culture organisms in the future, it is critical to carry out more research on the use of modified starter culture, mass production of starter organisms and give special attention for the cost of product.

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